

Application No. 10/577,861  
Amendment dated: March 2, 2009  
Response to Official Action of January 30, 2009

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

1. (Currently amended) An expression system for producing a B subunit of a cholera toxin (CTB) wherein the expression system comprises:  
*a Vibrio cholerae* host cell lacking the functionality of a *thyA* gene and a CTA gene; and an expression vector ~~less than 5kb in size having a size of 3 kb +/-20%~~ comprising a functional *thyA* gene and a CTB gene which is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived.
2. (Cancelled)
3. (Cancelled)
4. (Previously presented) The expression system according to claim 1 wherein the expression vector comprises an *E. coli thyA* gene.
5. (Previously presented) The expression system according to claim 1 wherein the expression vector has the nucleotide sequence presented in SEQ ID NO:1.
6. (Previously presented) The expression system according to claim 1 wherein the expression vector further comprises at least one further nucleotide sequence encoding a heterologous protein.
7. (Original) The expression system according to claim 6 wherein the further nucleotide sequence encodes a non-toxic component or form of the heat labile *E. coli*

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enterotoxin LT, preferably the non-toxic component of LT is the B subunit of a (LTB) or a fragment thereof.

8. (Currently amended) A method of producing CTB wherein the method comprises:

transforming a *Vibrio cholerae* host cell lacking the functionality of a *thyA* gene and a CTA gene with an expression vector less than 5kb in size having a size of 3 kb +/- 20% ~~and~~ comprising a functional *thyA* gene and a CTB gene which is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived,

and

culturing the transformed *V. cholerae* host cell under conditions which permit production of the CTB.

9. (Original) The method of claim 8 wherein the method further comprises isolating and/or purifying the CTB from the host cell.

10. (Currently amended) An isolated nucleic acid construct which comprises a *thyA* gene and a CTB gene which is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived, and which nucleic acid construct ~~is less than 5kb in size has a size of 3 kb +/-20%~~.

11. (Cancelled)

12. (Previously presented) The nucleic acid construct according to claim 10, wherein the nucleic acid construct is a plasmid.

13. (Original) The nucleic acid construct according to claim 12, wherein the plasmid is pMT-ctxB<sub>thyA</sub>-2 characterised by a restriction endonuclease map as shown in Figure 13.

14. (Original) The nucleic acid construct according to claim 12, wherein the plasmid has the nucleotide sequence SEQ ID NO: 1.

15. (New) An expression system for producing a B subunit of a cholera toxin (CTB) wherein the expression system comprises:

- c. a *Vibrio cholerae* host cell lacking the functionality of a *thyA* gene and a CTA gene; and
- d. an expression vector having a size of 3 kb +/-20% and comprising a functional *thyA* gene, a CTB gene fused to the LTB signal peptide from the heat-labile enterotoxin of *E.coli*; and which CTB gene is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived; and wherein the expression of the CTB gene is driven by the tac promoter and wherein a TrpA terminator is located downstream of the CTB gene.

16. (New) A method of producing CTB wherein the method comprises: transforming a *Vibrio cholerae* host cell lacking the functionality of a *thyA* gene and a CTA gene with an expression vector having a size of 3 kb +/-20% and comprising a functional *thyA* gene, a CTB gene fused to the LTB signal peptide from the heat-labile enterotoxin of *E.coli*; and which CTB gene is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived; and wherein the expression of the CTB gene is driven by the tac promoter and wherein a TrpA terminator is located downstream of the CTB gene.

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and

culturing the transformed *V. cholerae* host cell under conditions which permit production of the CTB.

17. (New) An isolated nucleic acid construct which comprises a *thyA* gene, a CTB gene fused to the LTB signal peptide from the heat-labile enterotoxin of *E.coli*; and which CTB gene is free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived; and wherein the expression of the CTB gene is driven by the tac promoter and wherein a TrpA terminator is located downstream of the CTB gene; and which nucleic acid construct has a size of 3 kb +/-20%.